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Mechanisms of Centaurea maculosa invasion in Montana Palouse Prairie and potential biocontrols

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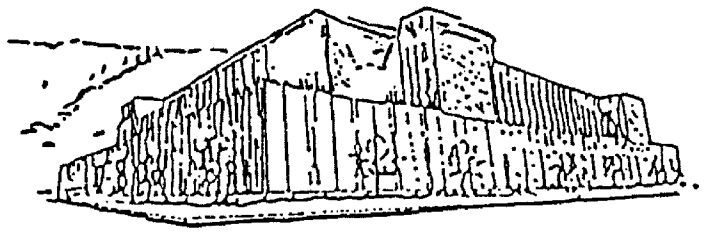

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Mechanisms of Centaurea maculosa Invasion in Montana Palouse Prairie and Potential Biocontrols

by

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Activated carbon, Allelopathy, Centaurea maculosa, Competition, Festuca idahoensis, Grasslands, Interference, Palouse prairie, Root interactions, Root-mediated allelopathy.

Director: Ragan M. Callaway

The relative roles of allelopathy and resource competition in plant interactions have been vigorously debated but seldom tested. I used activated carbon to manipulate the effects of root exudates on root elongation rates and the growth of neighbors in order to investigate the role of allelopathy in interactions between Centaurea maculosa Lam. and Festuca idahoensis Elmer. Festuca root elongation decreased after contacting Centaurea roots in sterile silica sand; however, contact with Centaurea roots had little effect on Festuca root elongation when activated carbon (which has a high affinity for adsorbing to organic compounds) was added to the sand. These results indicated that root exudates from Centaurea have the potential to harm Festuca. In pot experiments, Festuca plants were larger when grown with conspecifics than with Centaurea in pure silica sand. However, Festuca grown with Centaurea in mixtures of sand and activated carbon were significantly larger than when grown in pure silica sand. These results indicate that allelopathy is an important mechanism in the overall interfering effect of Centaurea on Festuca, and although Centaurea still outperformed Festuca in the presence of activated carbon, amelioration of Centaurea root allelopathy shifted the balance of competition in favor of Festuca.

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The Relative Effects of Allelopathy and Resource Competition between Centaurea maculosa and Festuca idahoensis

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Introduction

Plant interactions are often the products of complex combinations of specific mechanisms (Weldon and Slauson 1986; Callaway et. al., 1991). The general effects of competition are well documented (Connell 1983; Schoener 1983; Fowler 1986); however, competition for specific resources and its effect on plant fitness has rarely been demonstrated (but see Caldwell et al 1985, 1987; Ehleringer, 1984). The general role of allelopathy in plant-plant interactions is also well supported (Muller 1966; Muller et al. 1969; Rice, 1974; Mahall and Callaway, 1991, 1992). Although ecologists have identified the need to define competitive and allelopathic mechanisms more precisely (Harper, 1977; Connell, 1990; Williamson, 1990), understanding their relative importance in plant interactions has been hampered by the difficulties associated with separating the effects of each mechanism (Fuerst and Putnam, 1983). Despite a large body of experimental evidence that supports the existence of both of these mechanisms, only one study has examined the relative roles of resource competition and allelopathy between two species. Nilsson (1994) found that allelopathic leaf leachates of Empetrum hermaphroditum and general root interactions both contributed to the ability of Empetrum to suppress Pinus sylvestris seedling growth. However, the roles of root allelopathy and root competition were not separated in this study.

I examined the effects of allelopathic interference between Centaurea maculosa Lam. and Festuca idahoensis Elmer. in the context of general competitive interactions. Centaurea was introduced into North America from Eurasia early this century, and has

invaded natural Palouse prairie grasslands throughout the Pacific Northwest, decreasing the abundance and productivity of native species and reducing overall plant diversity (Muir and Majak, 1983; Rice et al., 1992). Centaurea invasion is often characterized by the development of dense monospecific patches. The mechanisms by which Centaurea displaces native species are poorly understood, but Fletcher and Renney (1963) suspected that Centaurea may be allelopathic, and used germination tests to assay extracts obtained from the leaves, stems, seeds, and roots of Centaurea to determine the presence of allelopathic substances. Kelsey and Locken (1987) isolated cnicin, a sesquiterpene lactone suspected of having allelopathic effects, from Centaurea roots and shoots and experimentally demonstrated its ability to inhibit seedling germination. The presence of potentially allelopathic chemicals, and the effects of extract assays in germination and growth experiments suggests the possibility of allelopathic interactions, but does not demonstrate their role in plant interactions. To the author's knowledge, no experiments have been conducted in which allelopathic effects (root exudates and leaf leachates) have been manipulated independently of resource competition.

I investigated the specific role of root allelopathy in overall plant-plant interference between Centaurea and the native Festuca by using activated carbon to reduce potential allelopathic effects in manipulative greenhouse experiments (root chamber and pot experiments), and asked following questions: 1) Does Centaurea interfere with Festuca via allelopathic root exudates? and 2) Does allelopathy play an important role in the overall interfering effect of Centaurea on Festuca?

Methods

To document field patterns suggestive of the competitive dominance of Centaurea, I sampled herbaceous vegetation in a natural Palouse prairie grassland that in some areas had been moderately to heavily invaded by Centaurea. Blocked randomized sampling was conducted in an area of uniform elevation, aspect, slope, and soil type. Sampling was conducted along 10-m transects using a 0.25 m^2 quadrat, and percent cover of all vascular plant species was visually estimated in areas of low Centaurea cover, in areas of moderate Centaurea cover, and in areas of high Centaurea cover.

I conducted greenhouse experiments during the period from March 1994 through December 1995 at The University of Montana. Greenhouse temperatures ranged from 20-30°C during the day, to 15-20°C at night. Supplemental high-pressure sodium lamps were used to maintain light between 600-800μmol m^{-2} s^{-1}. Centaurea and Festuca plants were started from seed in March and transplanted into root observation chambers designed after those of Mahall and Callaway (1991, 1992). Target and test plants of both species were planted into narrow rectangular containers (20.5 x 12.5 x 2 cm, inside dimensions), filled with a 50:25:25 mixture of 16, 20, and 30 grit pure silica sand and oriented at a 45° angle so that the positively geotropic roots grew down along Plexiglas viewing windows. These windows were covered with opaque shutters when roots were not being observed. After a period of establishment and growth in the chambers, pairs of chambers were connected

together so that the roots of "test" plants grew into the rhizosphere of "target" plants. This involved a 90° rotation of the chambers containing the target plants.

Thirty-eight pairs of root observation chambers were established. Twenty-four of these pairs contained Festuca test plants grown at Centaurea target plant rhizospheres, and 14 pairs containing both Festuca test and target plants were established as controls. We did not test Centaurea-Centaurea interactions. Of the 24 interspecific test/target combinations, 12 replicates included very finely ground activated carbon mixed evenly at 20 mL/L of sand in both test and target chambers. Carbon and no-carbon treatments were each replicated seven times for intraspecific (Festuca-Festuca) test/target control combinations. By thoroughly mixing finely ground activated carbon into the sand in half the root observation chambers, I established an experimental environment in which the effects of toxic root exudates and possible leaf leachates were reduced. Activated carbon has a high affinity for organic compounds, such as the suspected allelopathic toxins, and a weak affinity for inorganic electrolytes such as the nutrients in Hoagland's solution (Cheremisinoff and Ellerbusch, 1978) and has previously been shown to reduce the negative effects of root exudates from Larrea tridentata (Mahall and Callaway, 1991, 1992).

Elongation of Festuca test roots, approaching and contacting Centaurea or Festuca target roots in the environments described above were measured every other day through the viewing windows with a 6x magnifier (accurate to 0.1 mm). Measurements were taken before, during, and after Festuca test root contact with Centaurea or Festuca target rhizospheres. (Those Festuca test roots that did not make contact with Centaurea or

Festuca target roots are referred to as noncontact roots). These measurements yielded mm/hour Festuca test root elongation rates before, during, and after contact with Centaurea or Festuca target rhizospheres after calculations. Elongation rates of all roots were converted to mm/hour and standardized in time by aligning their days of contact at "day 0". Day 0 for noncontact roots was taken to be the average day of contact for sister contact roots of approximately the same age on the same plant. Treatments, then, were with and without activated carbon and with and without contact with target plant roots.

To investigate the role of allelopathy in the general interaction between Centaurea and Festuca, a total of 64 individual Festuca test plants were planted in one-L plastic pots filled with pure silica sand with either conspecific or Centaurea neighbors. Half of these test Festuca plants (32) were planted with Festuca neighbors (controls), and half were planted with Centaurea neighbors. Of these two neighbor species treatments, half of each (16) were planted in sand to which finely ground activated carbon had been mixed in at a rate of 20 mL/L sand, and half were planted in sand only. All plants were provided with plentiful quantities of deionized water three times per week, and nutrients (1/8th strength Hoagland's solution) once per week for the duration of the experiment. After 120 days, plants were harvested, divided into roots and shoots, dried at 60°C and weighed.

Because adding even small amounts of fine-grained carbon to the sand could alter water retention capabilities, I conducted an evapotranspiration experiment to test for differences between the two growth mediums. A total of 30 1-L pots were filled with sand, half of which contained pure sand and half of which contained sand to which finely ground activated carbon had been added at a rate of 20 mL/L sand. Of the 30 1-L pots

containing either carbon or no carbon growth medium treatments, five pots of each treatment contained no plants (evaporation only), five pots contained two Festuca plants, and five pots contained one Festuca and one Centaurea plant. These pots were all saturated with deionized water, weighed, placed in a greenhouse, and then weighed every four hours over a two day period (until the sand was mostly dry). Rates of evapotranspiration or evaporation were calculated and compared between carbon and no carbon treatments. A bioassay of the effects of activated carbon on the growth of Festuca was also conducted, wherein 25 1-L pots were filled with pure silica sand and 25 1-L pots were filled with sand into which finely ground activated carbon had been mixed at a rate of 20mL/L sand. All 50 1-L pots were planted with a single Festuca seedling. All plants were provided with plentiful quantities of deionized water three times per week and nutrients (1/8th strength Hoagland's solution) once per week throughout the experiment. After 90 days, plants were harvested, divided into roots and shoots, dried at 60°C and weighed.

Results

Vegetative sampling in a native Palouse prairie grassland that had been invaded by Centaurea revealed that after moderate Centaurea invasion, overall native plant diversity declined to 36% of that of uninvaded patches, and that heavy Centaurea invasion was characterized by almost monospecific stands of Centaurea (Table 1). Festuca cover

decreased by more than 75% with moderate Centaurea invasion, and was absent in heavily invaded areas.

The presence of activated carbon mixed into the sand significantly decreased the inhibitory effect of Centaurea target roots on the elongation rates of Festuca test roots (Figures 1 and 2). The average rates of elongation of Festuca test plant roots from four to six days prior to contact with Centaurea target plant roots did not vary significantly between carbon and no-carbon treatments (Table 2). However, two days prior to contact, rates of elongation of Festuca test roots that eventually contacted Centaurea target roots in no-carbon treatment chambers declined by more than 50%. Test Festuca roots continued to grow more slowly in the absence of activated carbon for the remainder of the experiment (eight days after contact). Elongation rates of Festuca noncontact test roots were also lower in no-carbon treatment chambers than in carbon treatment chambers (Table 3).

Festuca root growth, in general, was much less than that of Centaurea, thus intraspecific root contacts were rare and our sample size for Festuca-Festuca comparisons is low. However, Festuca test roots did not appear to be affected by conspecific contact and the responses of Festuca test roots to Festuca target roots did not appear to be affected by the presence of activated carbon. Elongation rates of Festuca test roots were the same with or without contact with Festuca target roots in both carbon and no-carbon treatments. Furthermore, Festuca test root survival was high when grown into Festuca target rhizospheres, and did not differ significantly between carbon and no-carbon treatments. When grown into Centaurea rhizospheres, Festuca test root survival was high

in carbon treatments only, and was significantly reduced in the no-carbon treatments (Figure 3).

Festuca plants were significantly larger when paired with conspecific neighbors, in carbon and no-carbon treatments (Two-way ANOVA, $F_{neighbor} = 10.35$, df = 1, $P = .002$) (Figure 4) indicating that Centaurea suppressed Festuca growth with or without fully functional allelopathic mechanisms. However, individual Festuca plants grown with Centaurea neighbors in pure sand were significantly smaller than Festuca plants grown with Centaurea neighbors in sand and activated carbon mixtures (Two-way ANOVA, $F_{carbon} = 6.11$, df = 1, $P = .017$) (Figure 4) indicating that activated carbon alleviated Centaurea's allelopathic effect on Festuca. Furthermore, Centaurea plants grown with Festuca neighbors in sand and activated carbon mixtures were significantly smaller than those grown with Festuca neighbors in sand only (Two-way ANOVA, $F_{carbon} = 5.81$, df = 1, $P = .018$) (Figure 5) indicating that activated carbon shifted the overall competitive balance in the favor of Festuca.

Evapotranspiration experiments, conducted to determine whether the addition of even small amounts of finely ground activated carbon to the sand could change the water holding characteristics of the sand, provided no evidence for such differences between carbon and no-carbon treatments. Furthermore, in the bioassay experiment, Festuca plants grown alone in pure sand were 20% larger than Festuca plants grown in sand with carbon (One-way ANOVA, $F_{carbon} = 4.85$, df = 1, $P = .033$).

Discussion

Root observation chamber experiments provided strong evidence for allelopathic effects of Centaurea root exudates on Festuca, and that these effects could be reduced by activated carbon. In pot experiments, the balance of competition shifted in favor of Festuca when effects of Centaurea root exudates, as well as possible leaf leachates, were ameliorated with activated carbon. Considered together, these experiments provide the first experimental evidence that allelopathy plays a role in the overall competitive balance between plants. Mahall and Callaway (1991, 1992) showed that root exudates of Larrea tridentata suppressed root elongation of conspecific and interspecific neighbors, but did not evaluate the role of this mechanism in the context of competition. Nilsson (1994) showed that allelopathic leaf leachates and general root interactions both contributed to the ability of Empetrum to suppress Pinus seedling growth, but did not separate the effects of root allelopathy and competition.

The specific chemical substance present in Centaurea root exudates responsible for its inhibitory effects on Festuca is unknown. Cnicin, a sesquiterpene lactone present in Centaurea has been suspected of being the allelopathic substance employed by Centaurea since 1987 (Kelsey and Locken, 1987). Allelopathy in Centaurea has been suspected since 1963 when Fletcher and Renney noticed that there is a tendency for invasive Centaurea species to form dense monospecific patches in which the establishment of native species is eliminated. They assayed extracts obtained from the

leaves, stems, seeds, and roots of three Centaurea species (C. maculosa, C. diffusa, and C. repens) on the germination of barley and lettuce to determine the presence of suspected allelopathic substances. Fletcher and Renney (1963) also found that the growth of tomato and barley plants was suppressed in soils taken from under dense patches of Centaurea and in soils taken from native grassland to which as little as 5 grams of dried, powdered Centaurea leaves were added. Muir and Majak (1983) found that seed germination and seedling growth of ryegrass were inhibited when extracts from Centaurea leaves were added to petri dishes containing germination pads and ryegrass seeds. They discovered that the inhibitory effects were associated with those fractions of extracts containing sesquiterpene lactones. Sesquiterpene lactones have been experimentally demonstrated to inhibit IAA-induced elongation growth of plants (Spring and Hagar, 1982). Kelsey and Locken (1987) used column chromatography along with a bioassay of Centaurea shoot extracts with lettuce seeds to isolate cnicin, a sesquiterpene lactone. Pure cnicin was then assayed at varying concentrations against the seeds of lettuce, Centaurea, and several native species. Cnicin was found to significantly retard seedling growth. Bioassays of suspected volatile phytotoxins or allelopathic leachates (extracts) do not, however, eliminate alternative mechanistic explanations, as they do not demonstrate that these toxic substances either accumulate at sufficient concentrations or persist long enough under normal growing conditions to inhibit the growth or development of other plants. My results with Centaurea directly demonstrate the occurrence of root mediated allelopathy as a mechanism of interference under controlled conditions, but these processes may differ in complex, natural field systems.

Root-mediated allelopathy has been suspected since the early 1800's, when De Candolle (1832) suggested the possibility that plants may exude substances from their roots detrimental to the growth of other plants. Others have also used activated carbon to manipulate the effects of root exudates on the growth of other plants. Early this century, Schreiner and Reed (1907, 1908) argued that substances deleterious to plant growth were excreted into the soil by growing roots and found that such soil loses its toxicity with the addition of "carbon black". Mahall and Callaway (1991,1992) found that Larrea roots allelopathically inhibit elongation of nearby Larrea or Ambrosia roots. The presence of activated carbon caused a significant decrease in the ability of Larrea roots to inhibit the elongation of neighboring roots. Krannitz and Caldwell (1995) used techniques similar to mine to study inter- and intraspecific root interactions between Artemesia tridentata, Pseudoroegneria spicata, and Agropyron desertorum. They found that contact with Agropyron roots caused Pseudoroegnneria roots to stop growing.

The overall effect of one plant on another is the result of multiple interacting mechanisms (Callaway, 1995; Chapin et al., 1994; Nilsson, 1994). By manipulating allelopathic mechanisms (I mixed activated carbon into the entire soil column, reducing allelopathic interference so that only resource competition was left in the overall interfering interaction between Centaurea and Festuca), I established that Centaurea roots allelopathically inhibited elongation of nearby Festuca roots under controlled conditions and that the general balance of competition shifted when allelopathy was ameliorated. Continued study of the importance of allelopathy in the context of general competitive

effects and relative to competition for specific resources may go far to depolarize current perspectives on allelopathy.

Acknowledgments

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Tables

Table 1. Centaurea invasion and community diversity: percent cover of Centaurea and other herbaceous species in a natural Palouse prairie grassland that in some areas has been moderately to heavily invaded by Centaurea. Sampling was conducted along 10-m transects using a 0.25 m^2 quadrat, and percent cover of all vascular plant species was visually estimated in areas of low Centaurea cover, in areas of moderate Centaurea cover, and in areas of high Centaurea cover.

	Native	Light Invasion	Heavy Invasion
Species and percent cover			
Centaurea maculosa	1.4	49.3	100.0
Festuca idahoensis	16.1	3.4	0
Lupinus sericeus	3.9	5.8	1.4
Festuca scabrella	2.1	2.3	0
Pseudoroegenria spicata	11.0	0.9	0
Koeleria cristata	0.8	0	0
Aster falcatus	4.0	0	0
Balsamorhiza sagittata	3.7	0.1	0
Total native plant richness	15	16	1
Total native plant diversity	1.56	0.1	0.06

Table 2. Elongation of Festuca test plant roots growing toward Centaurea or Festuca target roots from four to six days prior to contact, in root observation chambers with or without activated carbon. Interactions were not significant.

Effect	df	F	P
Carbon	1	0.06	.807
Species	1	3.27	.076
Contact*	1	0.46	.502

* Root that eventually made contact with target roots.

Table 3. Elongation of Festuca test plant roots growing toward Centaurea or Festuca target roots from two days prior to contact until eight days after contact, in root observation chambers with or without activated carbon. Interactions were not significant.

Effect	df	F	P
Carbon	1	22.4	<.001
Species	1	7.3	.009
Contact	1	9.8	.003

Figures

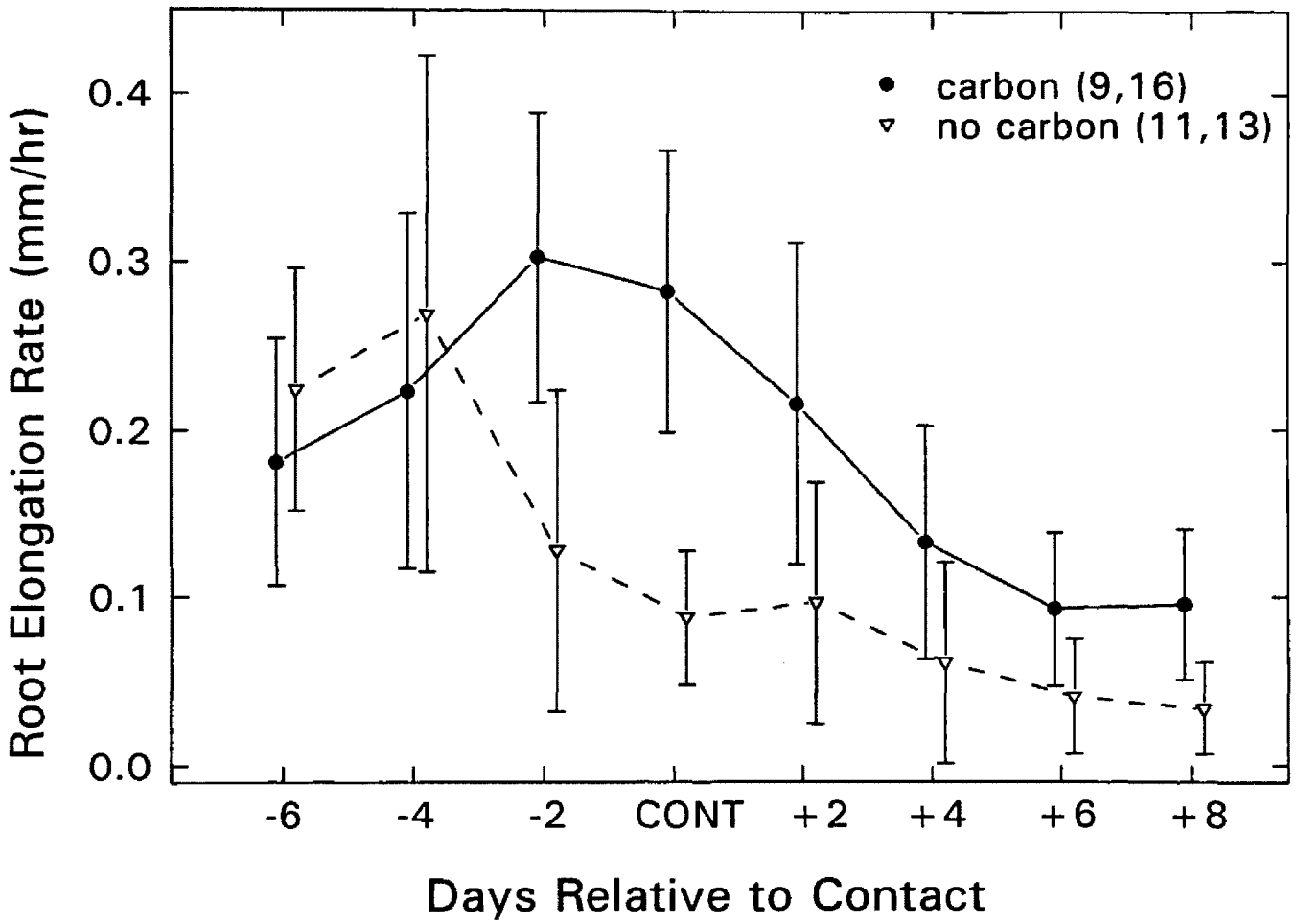
Figure 1. Elongation rates of Festuca contact roots when grown towards Centaurea roots in root observation chambers, with or without activated carbon, from six days before until eight days after contact. Elongation rates of all roots were converted to mm/hour and standardized in time by aligning their days of contact at "day 0". Error bars represent two standard errors on both sides of the mean.

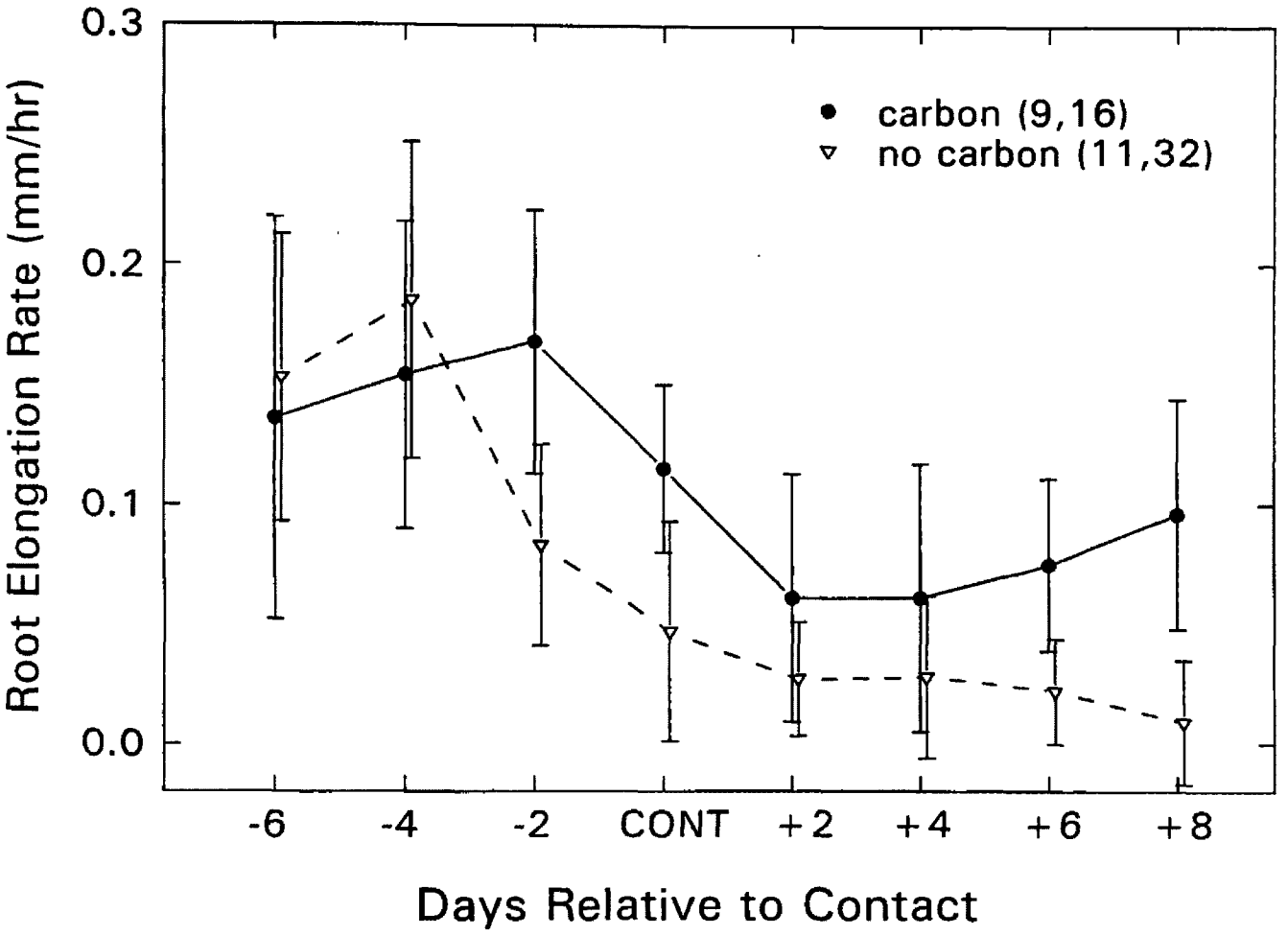
Figure 2. Elongation rates of Festuca noncontact roots when grown towards Centaurea roots, with or without activated carbon, from six days before until eight days after contact. Elongation rates of all roots were converted to mm/hour and standardized in time by aligning their days of contact at "day 0". Day 0 for noncontact roots was taken to be the average day of contact for sister contact roots of approximately the same age on the same plant. Error bars represent two standard errors on both sides of the mean.

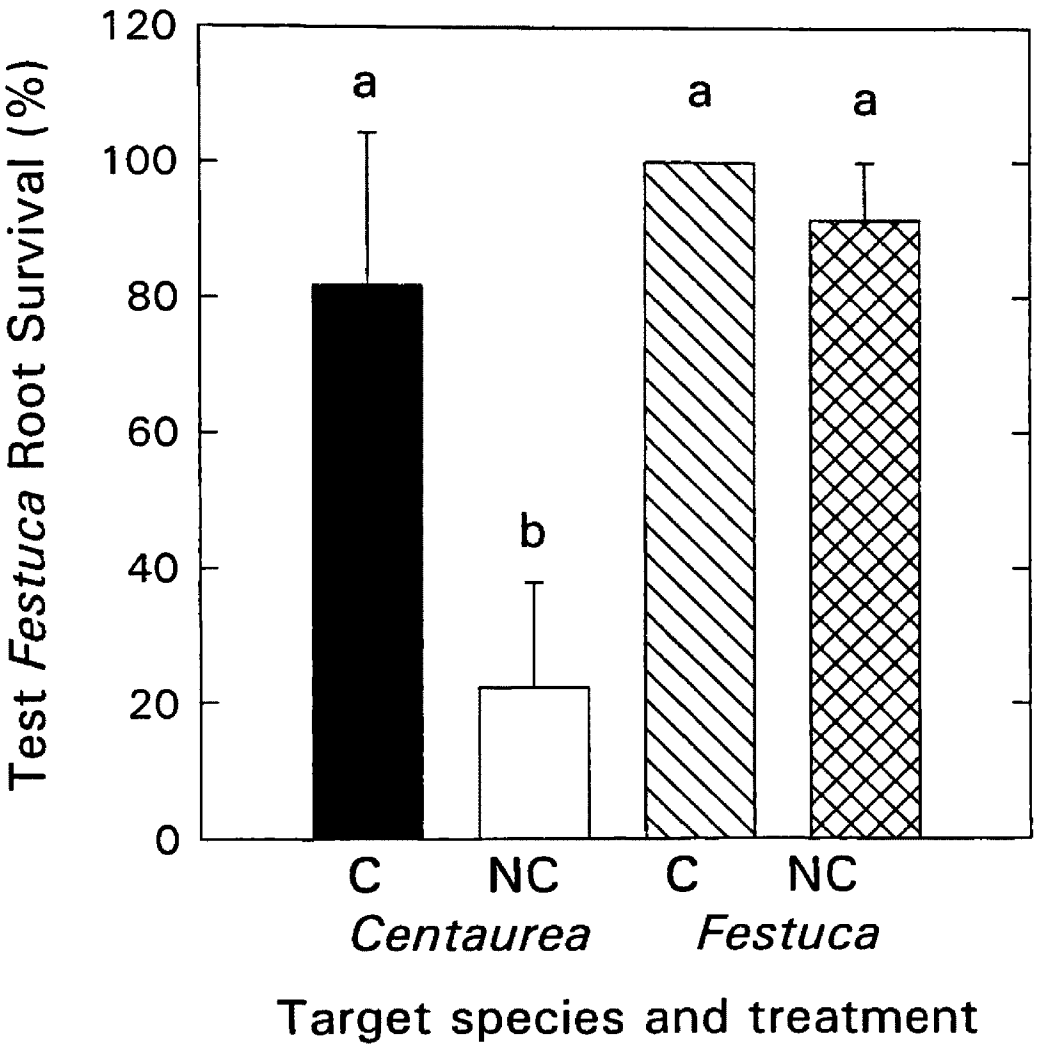
Figure 3. Test Festuca root survival when grown with Centaurea or Festuca neighbors in root observation chambers with or without activated carbon. Error bars represent two standard errors on both sides of the mean.

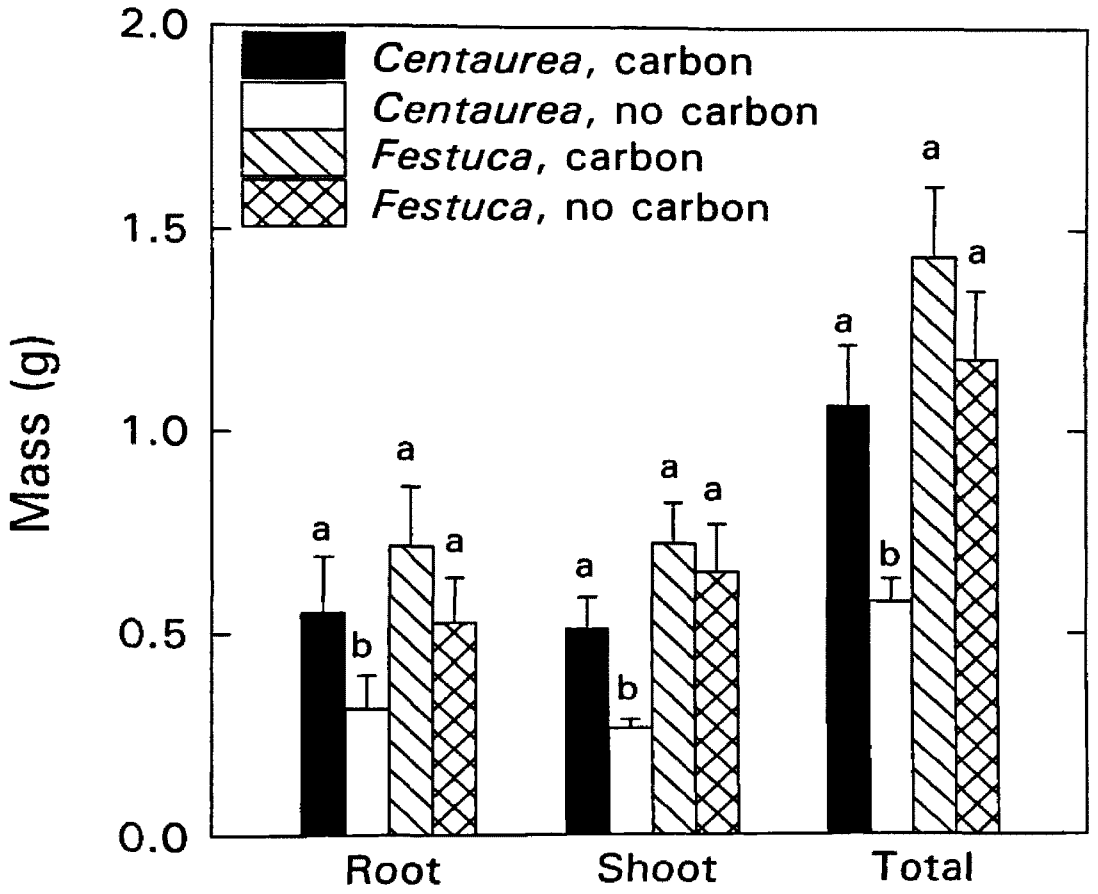
Figure 4. Biomass of test Festuca plants grown with Centaurea or Festuca neighbors in pots with or without activated carbon. Error bars represent two standard errors on both sides of the mean.

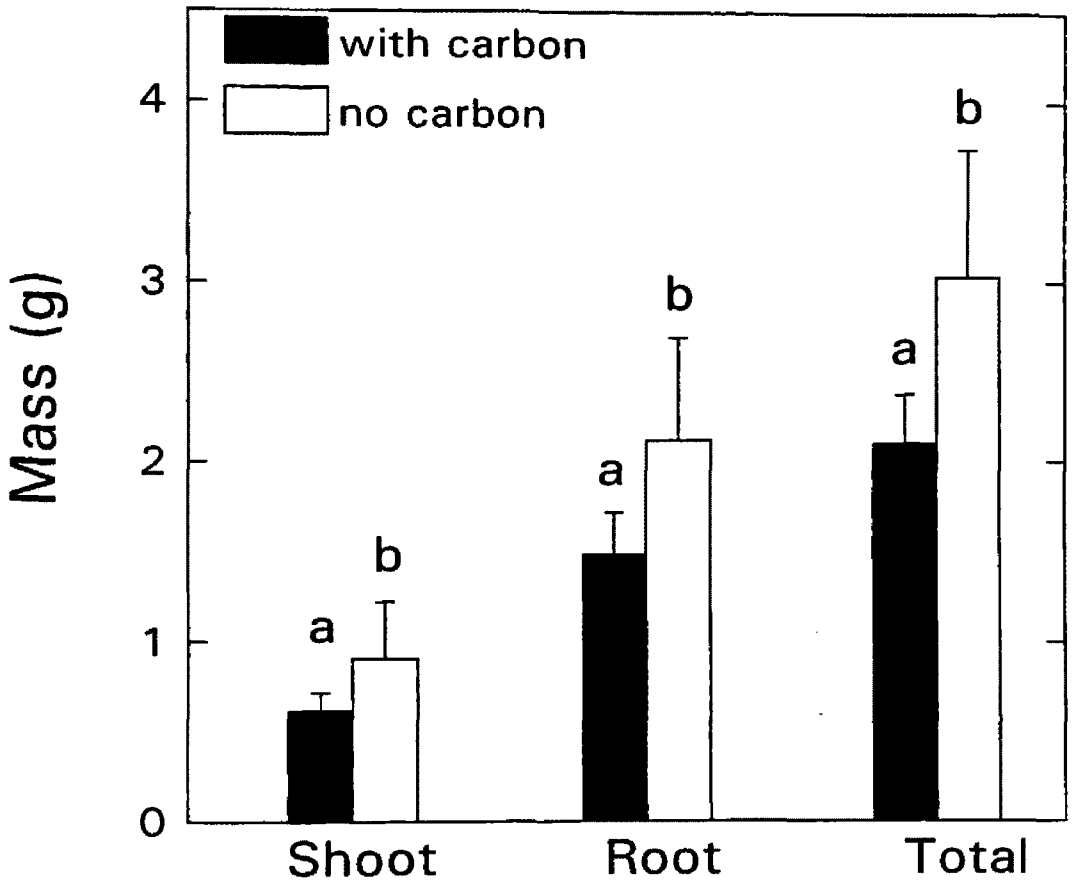
Figure 5. Centaurea biomass when grown with Festuca neighbors with or without activated carbon. Error bars represent two standard errors on both sides of the mean.











Root Herbivores, Pathogenic Fungi, and Competition Between an Exotic and a Native Species

Ridenour, Wendy L., M. S., December 1995 Division of Biological Sciences

Agapeta zoegana, Biological control, Centaurea maculosa, Common garden experiments, Competition, Festuca idahoensis, Grasslands, Interference, Palouse prairie, Sclerotinia sclerotiorum.

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Direct interactions between two species can be altered by the presence of other species. I used common garden experiments to evaluate the effects of multiple, or higher order interactions in the biocontrol of the exotic plant Centaurea maculosa Lam., one of the most aggressive invaders in the northwestern United States. Competitive interactions between Centaurea and the native bunchgrass Festuca idahoensis Elmer. were studied in the presence and absence of the root-mining herbivore Agapeta zoegana (associated with the roots of Centaurea in Europe), and a native soil-borne fungal pathogen, Sclerotinia sclerotiorum. To explore these interactions, I grew Centaurea and Festuca in a common garden experiment over a two year period in six different treatments: 1) Centaurea control, 2) Festuca control, 3) Centaurea x Festuca treatment, 4) Centaurea x Festuca with Agapeta treatment, 5) Centaurea x Festuca with Sclerotinia treatment, and 6) Centaurea x Festuca with Agapeta and Sclerotinia treatment. Centaurea strongly interfered with Festuca. Sclerotinia killed all Centaurea in plots that received this treatment (Centaurea x Festuca with Sclerotinia and Centaurea x Festuca with Agapeta and Sclerotinia), and Festuca growth and reproduction was equal to that in pure Festuca plots. Agapeta did not affect the interaction between Centaurea and Festuca in this experiment either alone or in combination with Sclerotinia. My study showed that native pathogens have the potential to control invasive plants by altering the direct competitive hierarchy, but I found no evidence for additive or synergistic effects of combined biocontrols.

Introduction

Inter-plant interactions can be highly altered by herbivory and pathogens (Louda et al., 1990; Clay, 1990) and these higher order interactions have important consequences for the control of exotic, invasive plants. However, there have been relatively few controlled, experimental studies of the effects of consumers on competition between exotic and native plants (Clay, 1990; Clay et al., 1993). Furthermore, almost all studies of the effects of herbivores and pathogens on inter-plant interactions have focused on a single consumer species, even though the effects of multiple species assemblages cannot be predicted from paired experiments (Adler and Morris, 1994; Kareiva, 1994; Miller, 1994; Wootton, 1994). Many biological controls have been introduced for the potential biological control of Centaurea maculosa Lam., and a native fungus, Sclerotinia sclerotiorum, infects Centaurea; thus, the Palouse prairie has effective native competitors, native pathogens that damage Centaurea, and insect herbivores introduced as Centaurea biocontrols. However, these agents have not been used in experiments designed to compare the relative efficacy of simple versus higher order interactions.

Application of multiple, or higher order interactions, may contribute to successful biological control of invasive plant species, as biological control organisms, including competitors, herbivores, and pathogens may elicit different effects in combination than in isolation. The Palouse prairie grasslands of western Montana are an ideal system in which to investigate the importance of multiple interactions in the biocontrol of invasive plant species. Although a diverse native flora remains, much of this system has been invaded by

exotics including Centaurea maculosa, a biennial or short-lived perennial that was introduced into North America from Eurasia. Centaurea is one of the most widespread grassland invasives in the Western United States and Canada (Griffith and Lucey, 1991), and its negative effects on species diversity have been well documented (Rice et al., 1992). In some locations, productivity and diversity of other grassland species has been reduced by more than 90% by Centaurea species (Muir and Majak, 1983; Ridenour and Callaway, in review).

I studied the isolated and combined effects of two consumers (Sclerotinia and Agapeta) on Centaurea, and focused on the following question: Can the combined effects of fungal and insect consumers on the balance of competition between Centaurea and the native Festuca idahoensis Elmer. be predicted from their separate effects? I pursued answers to this question by using manipulative common garden experiments.

Methods

I conducted common garden experiments at The University of Montana Diettert Experimental Gardens. These gardens occupy land once covered by Palouse prairie, and are nearby natural Palouse prairie grasslands that have been heavily invaded by Centaurea. Centaurea and Festuca plants were started from seed in March in The University of Montana greenhouse and transplanted into garden plots in May. The experimental design consisted of six treatments, each of which was replicated ten times for a total of sixty 0.25 m^2 plots that were randomly located in the garden. Plots were placed 0.50 m apart, and

plants were located ten cm from neighbors. Treatments were as follows: 1) Centaurea control, 2) Festuca control, 3) Centaurea x Festuca treatment, 4) Centaurea x Festuca with Agapeta treatment, 5) Centaurea x Festuca with Sclerotinia treatment, and 6) Centaurea x Festuca with Agapeta and Sclerotinia treatment. Ten control plots were established in which the intraspecific effects of Festuca were measured. In each case, twenty-four plants were planted in each 0.25 m^2 plot so that each plant was 10 cm from all neighbors. Ten control plots were also established in which the intraspecific effects of Centaurea were measured. Ten Centaurea x Festuca treatment plots were also established in which both Centaurea and Festuca were alternatingly planted in order to quantify the effects of Centaurea on Festuca. This allowed for the comparison of the growth of Festuca plants experiencing intraspecific competition to growth of Festuca experiencing competition from Centaurea to define the competitive relationship between these plants.

To investigate the relative effects of treatments on soil water availability, PVC monitoring tubes were installed 30 cm deep in the center of each of the 60 garden plots. Soil moisture was measured using Frequency Domain Reflectometry (Troxler, Sentry 200-AP) once per week at 15 cm and 30 cm depths beginning in April and ending at the end of October 1994 and 1995. This compared the effects of Centaurea on soil water availability in monocultures, mixed stands, and in mixed stands with biocontrols.

To investigate the relative effects of treatments on soil nutrient availability, ion exchange resin bags were used to accumulate available nitrogen and phosphorous during the first year of the study only (1994) (Binckley and Vitousek 1991). Resin bags were buried 15 cm deep near the center of each of the 60 garden plots. These bags were used

to evaluate available soil nitrogen and phosphorous for the period April 15 - October 15, 1994. Nitrogen was measured as 1 molar KCl extractable ammonium and nitrate, and phosphorous was measured as 1 molar KCl extractable phosphorous. At the end of the 1994 growing season, resin bags were removed from each plot, and their extracts were colorimetrically analyzed for available nitrate, ammonium, and phosphorous.

Two biocontrol treatments were applied, both singly and in combination with one another. These biocontrols were the lepadopteran root-mining herbivore Agapeta zoegana and the fungal pathogen Sclerotinia sclerotiorum. Agapeta was introduced into the United States for the biocontrol of Centaurea. Adult insects deposit their eggs on Centaurea plants, and the larvae then mine Centaurea roots. Agapeta were acquired from the Montana State University Agricultural Experimental Station in Corvallis, Montana. Ten plots, identical in layout and composition to the Centaurea x Festuca treatment plots were established and infected with Agapeta to determine whether or not root herbivory would shift the balance of competition in the favor of Festuca. After a period of establishment and growth, fine mesh cages were placed over all plots and three adult Agapeta were introduced into each of the ten Agapeta treatment plots. Cages were placed over all of the other plots to control for the effects of caging. After ten days, the time allowed for egg-laying, cages were removed from all plots.

Sclerotinia is a native soil-borne fungus that was acquired from the lab at Montana State University, where it is being developed as a biocontrol agent. Ten plots, identical in layout and composition to the Centaurea x Festuca treatment plots, were established and infected with Sclerotinia by applying 2 cm^3 of Sclerotinia-infected grain to the base of each

Centaurea stem to determine whether or not fungal infection would shift the balance of competition in the favor of Festuca.

In order to test the combined effects of the consumers, ten plots, identical in layout and composition to the Centaurea x Festuca treatment plots were established and infected with both Agapeta and Sclerotinia. All biocontrols were applied within a two- day period.

In October of 1994, volumetric measurements and number of green leaves (fall green-up) of four equally positioned Festuca plants in each plot were collected.

In October 1995, all surviving plants were harvested, dried at 60°C, and weighed to obtain measures of above ground biomass. A count of Festuca florets per plot was also made at this time to obtain measures of Festuca reproductive output.

Results

All of the Centaurea plants in both Sclerotinia treatments (Centaurea x Festuca with Sclerotinia and Centaurea x Festuca with Agapeta and Sclerotinia) died within two weeks of application. Centaurea biomass in the remaining Centaurea plots (Centaurea control, Centaurea x Festuca, and Centaurea x Festuca with Agapeta) did not vary significantly between treatments (One-way ANOVA, $F_{treatment} = 0.948$, df = 5,59, $P = 0.400$) (Figure 1). Total Centaurea biomass / m^2 was not affected by the presence of Festuca, even though initial Centaurea planting densities differed among treatments (Figure 1).

The competitive effect of Centaurea on Festuca was pronounced. Total Festuca biomass in the Festuca control plots was significantly greater than in the Centaurea x Festuca treatment plots (One-way ANOVA, $F_{treatment} = 4.32$, df = 5,59, $P = 0.003$) (Figure 2). Festuca floret production was also significantly greater within Festuca control plots than in the Centaurea x Festuca treatment plots (One-way ANOVA, $F_{treatment} = 8.368$, df = 5,59, $P = 0.000$) (Figure 3). The competitive effect of Centaurea on Festuca, however, was not manifest through competition for soil water. The soil moisture content at 15 and 30 cm depths did not vary significantly between treatments in either year (Repeated-measures ANOVA, $F_{treatment \times time\ at\ 15\ cm} = 0.93$, df = 5,59, $P = 0.606$; $F_{treatment \times time\ at\ 30\ cm} = 1.04$, df = 5,59, $P = 0.412$). Likewise, the competitive effect of Centaurea on Festuca was not manifest through competition for soil nutrients. Available soil nitrate, ammonium and phosphorous did not vary significantly between treatments during the first year of the study (One-way ANOVA, NO_3: $F_{treatment} = 0.511$, df = 5,59, $P = 0.767$; NH_4: $F_{treatment} = 0.446$, df = 5,58, $P = 0.814$; P: $F_{treatment} = 1.20$, df = 5,59, $P = 0.321$).

In isolation, the root-mining herbivore Agapeta (Centaurea x Festuca with Agapeta) had no effect on either Centaurea or Festuca biomass (Figure 1). In contrast, all Centaurea plants in Sclerotinia treatment plots (Centaurea x Festuca with Sclerotinia and Centaurea x Festuca with Agapeta and Sclerotinia) died within two weeks of application (Figure 1). Elimination of Centaurea had significant effects on the growth of Festuca (Figures 1 and 2). By October 1994, individual Festuca plants in the Sclerotinia treatment plots were as large as those in the Festuca controls. In the controls, Festuca density was greater, and thus intraspecific competition was probably greater. By October 1995,

above-ground Festuca biomass / m^2 and floret production in the Sclerotinia treatment plots were not significantly greater than that in Festuca controls (Figures 1, 2, and 3).

Because of the strong effect of the fungal consumer on Centaurea (directly) and Festuca (indirectly), and the absence of an effect of the herbivore, no combined higher order effects occurred.

Discussion

Common garden experiments supported general observations that Centaurea has strong direct interfering effects on Festuca, but Festuca was not competitively excluded from experimental plots. Sclerotinia can dramatically alter the balance of this interaction by killing Centaurea and releasing Festuca. However, Agapeta, an herbivore imported for the express purpose of controlling Centaurea, did not have an effect on Centaurea or Festuca biomass or their interactions in this experiment. In a study of Agapeta, Centaurea, and Festuca pratensis (commonly associated with Centaurea in Europe) in their native habitat, Muller-Scharer (1991) found that competition from Festuca pratensis resulted in a more pronounced herbivore impact on the reduction of Centaurea plant height than did herbivory alone. He also found that "in the absence of grass competition, herbivory showed no significant impact on plant height, biomass, and fecundity" (Muller-Scharer, 1991). In fact, Muller-Scharer found that grass competition seemed to be the single most important factor influencing the results of Agapeta herbivory of Centaurea (Muller-Scharer, 1991). It is possible that Festuca pratensis, being commonly associated

with Centaurea in Europe, is a stronger competitor with Centaurea than is Festuca idahoensis, which may explain some of the contradictory results between the two studies. It is also possible that a higher density of adult Agapeta is necessary to obtain a resultant shift in the balance of the Centaurea-Festuca interaction. While Muller-Scharer's study of the interactions between herbivory and plant competition among Agapeta, Festuca pratensis, and Centaurea demonstrates that competition with other plants can increase the negative effects of selective herbivory, our study demonstrates that pathogens such as Sclerotinia can change the ability of a plant to compete with other plants.

My common garden experiments also suggest that the interfering effect of Centaurea on Festuca were not manifest through depletion of soil resources (soil water, or available soil nitrate, ammonium, or phosphorous). Results of previous experiments with Centaurea and Festuca indicate that allelopathic root exudates are important in the overall interfering effect of Centaurea on Festuca, and may explain why competition for soil resources did not appear to be important in my common garden experiments (Ridenour and Callaway, in review).

Classical biological control can be defined as the introduction of the natural enemies of an invasive plant from that plant's natural environment in order to control its spread (Harris, 1991). Most biological control efforts have made use of the release of one biological control agent (usually an insect herbivore or a fungal or viral pathogen) (Wurtz, 1995; Julien, 1992). This strategy has sometimes proven successful (Harris, 1984). My study clearly demonstrates that the introduction of a fungal pathogen such as Sclerotinia can, under carefully controlled conditions, markedly shift the balance of competition away

from the favor of an invasive plant such as Centaurea and towards the favor of a weaker competitor such as Festuca. Even though my experiments found an overwhelming effect of a single consumer, and thus no complex higher order effects, I suggest that studies of multiple or higher order interactions should be considered in the biological control of invasive plant species. Pathogens and herbivores may alter the balance of competition in plant communities, and certain plant competitors may affect the degree of the response of a competing plant species to selective herbivores or pathogens. Community organization, then, or the combined higher order interactions between organisms from different trophic levels, must be considered in biocontrol efforts, as the effects of herbivores or pathogens cannot be considered as simple additive components regardless of community structure. As such, community structure may be an important factor in the regulation of an invasive plant species, as herbivores or pathogens acting alone may be insufficient in the control of an invasive plant species. Both herbivores, pathogens, and competitors can slow plant growth, reduce plant biomass, reduce plant reproductive output, and reduce plant survivorship, thus altering plant position in competitive hierarchies.

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Figures

Figure 1. Above-ground per plot biomass of Centaurea and Festuca when grown in a common garden experiment in six different treatments. Error bars represent two standard errors on both sides of the mean.

Figure 2. Above-ground per plot biomass of Festuca for six different treatments. Error bars represent two standard errors on both sides of the mean.

Figure 3. Festuca floret production per plot for six different treatments. Error bars represent two standard errors on both sides of the mean.